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EUROPEAN PATENT APPLICATION

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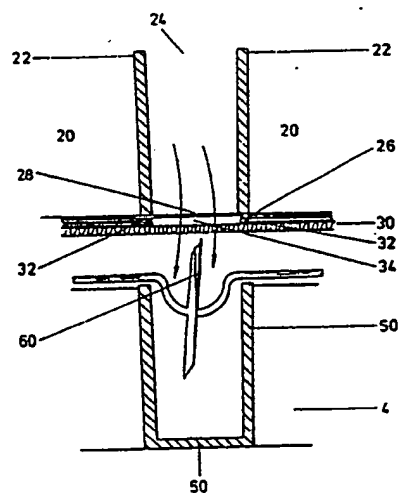
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⑧④ Designated Contracting States: CH DE FR GB LI SE

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⑤④ Filter apparatus.

⑤⑦ A multiwell filtration apparatus for the assay of microliter quantities is provided which prevents fluid loss by capillary action and gravity flow through a microporous membrane or ultrafilter. The filtration apparatus is particularly advantageous in assays requiring maintenance of fluid within the reaction wells for substantial time periods and in small sample volume assays in the range of 100 microliter volumes.



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1 **PATENTANWALTE**
 Z E L L E N T I N
 ZWEIBRÜCKENSTR. 15
 8000 MÜNCHEN 2

5 Millipore Corporation June 30, 1983
 Bedford Massachusetts 01730 Eu 83 223 AS/K
 U.S.A.

Filter Apparatus

10

 The invention relates to laboratory apparatus useful
 in the assay of biological and biochemical reactants
 and is particularly concerned with multiwell filtration
15 devices able to retain fluids for substantial periods
 of time before filtration is performed.

20 Test plates for in vitro analysis which contain a
 multiplicity of individual wells or reaction chambers
 are commonly known laboratory tools. Such devices have
 been employed for a broad variety of purposes and assays
 as are exemplified by U.S. Patent Nos. 3,649,464;
 4,304,865; 4,276,048; 4,154,795; and Re 30,562. Micro-
25 porous membrane filters and filtration devices containing
 such microporous membranes have become especially useful
 with many of the recently developed cell and tissue
 culture techniques and assays - particularly those in
 the fields of virology and immunology.

30

 Typically, a 96-well filtration plate is used to conduct
 multiple assays simultaneously some of which last
 several hours before filtration is actually performed.
 With such filtration plates, especially those containing
35 microporous membranes, there is a well recognized and
 recurrent problem in that fluids in the wells tend to
 pass through the membrane by capillary action and gra-

1 vity flow thereby causing a loss of contents from
within the reaction well before the desired stage in
the experimental design. Prevention of fluid loss by
capillary action and gravity flow becomes especially
5 important when living cells or tissues are being
maintained or grown within the reaction wells. Under
these circumstances, favorable media conditions for the
cells or tissues must be maintained for hours or even
days and any loss of fluid from the wells, however
10 small, will affect the condition of the cells and
influence the results of the assay. Prevention of fluid
loss through the membrane in this manner is also vitally
important when the assay utilizes very small sample
volumes as reactants, such test samples often being
15 less than 100 microliters in volume. The pendant drop
that invariably forms on the underside of the micro-
porous membrane due to such capillary action and gravity
flow is typically about 50 microliters in volume and it
is apparent that a fluid loss of such proportions must
20 drastically affect the assay.

Nevertheless, insofar as is presently known, no filtra-
tion apparatus has been able to prevent this loss of
fluid from the reaction well, particularly under small
25 sample volume assay conditions.

A filtration apparatus for the assay of microliter quan-
tities of biological and biochemical reactants is
30 provided comprising a plate having a plurality of
apertures open at each end, filtration means disposed
across and sealed about one end of each aperture
thereby forming a well with a discrete filtering area
and a hydrophobic fabric disposed across a bonded
35 adjacent to the filtering area bounded by each well.

1 The hydrophobic fabric prevents a loss of fluid by
capillary action and gravity flow from within the well
in the absence of an applied differential pressure.
Additionally provided are fluid collection means and
5 a guiding projection which directs such fluid as passes
through the filtration means to a predetermined location
within the fluid collection means.

10 The present invention may be best understood when taken
in conjunction with the accompanying drawing, in which:

Fig. 1 is an expanded view of a vacuum assembly useful
with the invention;

15

Fig. 2 is an overhead view of a filtration apparatus
comprising one embodiment of the present invention;

20 Fig. 3 is a cross-sectional view of the preferred
filtration apparatus comprising the present invention;

Fig. 4 in one embodiment of fluid collections means
useful with the preferred embodiment illustrated in
Fig. 3; and

25

Fig. 5 is another preferred embodiment of the invention
illustrated in Fig. 3.

30 The invention is an improvement in filtration apparatus
having at least one reaction well which typically
contains a microporous membrane for the separation
and retention of matter from fluids. Attached adjacent
to the microporous membrane is a porous hydrophobic
35 fabric which is situated either above or preferably

1 below the filtering microporous membrane. This hydro-
phobic fabric prevents fluid loss by capillary action
and gravity flow through the membrane in the absence
5 of a vacuum force but will still allow diffusion of
gases into or out the interior of each well on the
plate.

Embodiments of the invention are most useful with the
10 vacuum assembly shown in Fig. 1 which is capable of
simultaneously processing 96 individual test samples
of up to 440 microliters (μ l) each. The vacuum assembly
comprises a base 2 which acts as a vacuum chamber and
contains a hose barb for connection to a regulated
15 external vacuum source. Housed within the base 2 are
fluid collection means 4 which include a collection
try 6 and/or a receiving plate 8 having up to 96
individual chambers for the collection of filtrate. A
filter support 10 holding a 96-well filtration plate 12
20 lies above the fluid collection means 4 separated by
gaskets 14 and 16 which form an airtight seal in the
presence of a vacuum force.

Detailed views of the filtration plate utilizing the
25 preferred embodiment of the present invention are
shown in Figs. 2 and 3. It will be appreciated that
the number of wells found in the filtration plate
are simply a matter of convenience for the investigator.
The plate 20 may contain as few as one well or as many
30 wells as are functionally permissible given the actual
dimensions of the plate. The filtration plate may be
formed of any resilient and nonreactive material
commonly available, the composition of choice being a
matter of convenience or economics only. Each well 22
35 comprises an aperture 24 through the entire depth of
the plate, the thickness of the plate determining the

1 volume of fluid to be retained within the well. The
diameter of the aperture will vary to meet the user's
needs but typically will range from 3 to 25 millimeters
5 in diameter. The filtration means 26, typically a
microporous membrane filter, is disposed across and
sealed about the aperture 24 in the plate 20 such that
the area across each well will serve as a filtering
area 28. Methods of bonding the microporous membrane
10 to the plate and sealing it about the perimeter of the
aperture 24 are well known in the art and need not be
described in detail here. The composition and flow
characteristics of the filtration means 26 forming
the filtering area 28 across each aperture 24 is also
15 a matter of choice. Typically nitrocellulose membranes
cellulose acetate, polycarbonate and polyvinylidene
fluoride microporous membranes are selected because
of their proven characteristics in aqueous solutions
and tissue culture media. The porosity of the membrane
20 will be selected with a view to the chosen application.
Although 0.025 to 10.0 micrometer porosity membranes
of 150 micrometers thickness are favored, the filtra-
tion means 26 are not limited to microporous membranes
as such. Rather, ultrafiltration media can be utilized
25 in lieu of microporous membrane. By the term ultra-
filtration media is meant a material capable of re-
taining a molecule in solution. Such ultrafiltration
media are useful for retaining molecules as small as
about 100 daltons and generally molecules as large
30 as about two million daltons. Examples of such ultra-
filtration media are well known in the art and include
polysulfone and other polymeric materials available
from Millipore Corporation under the registered
trademark, PELLICON[®]. Similarly, macrofiltration media
35 such as glass fiber for retention of gross particles
may be used. It will be appreciated by those ordinarily

1 skilled in the art that the individual filtering
areas 28 bounded by each well 22 can be removed via
a filter punch after filtration for further processing
5 if necessary.

As can be seen in Fig. 3, a hydrophobic fabric 30 is
disposed across and bonded adjacent to the filtering
areas 28 of the well 22. Preferably, the hydrophobic
10 fabric is bonded to the filtration means abutting the
well perimeter 32 such that a minute space 24 is
created and maintained between the fabric 30 and the
filtering area 28. The fabric 30 may be heat bondable
or utilize an adhesive for attachment to the filtration
15 means 26. In addition, the fabric 30 may be formed of
woven or a nonwoven materials and be composed any
of hydrophobic polyester, polyolefin, polytetrafluoro-
ethylene or other polymer - many suitable varieties
being commercially available.

20 It is preferred that attachment of the filtration means
26 and the hydrophobic fabric 30 to the plate 20 be
performed as separate steps to insure their proper
positioning and the formation of the minute space 34.
25 Nevertheless, it is possible to attach both the fil-
trations means and the hydrophobic fabric simultaneously,
particularly if a heat bondable hydrophobic material
is used as the fabric layer.

30 Affixation of a porous hydrophobic fabric in this manner
permits the use of small sample volumes, often less
than 100 microliter (hereinafter μ l), to be used as
reactants. Without the fabric layer, a drop of fluid
approximately 50 μ l in volume will collect below the
35 filtration means as a pendant drop and become lost.
With the hydrophobic fabric in place, the pendant

1 drop that forms below the filtering area 28 as a result
of capillary action and gravity flow will be substanti-
ally retained within minute space 34 and the tendency
5 for liquid to pass through the filtering area is
substantially reduced or entirely eliminated. As a result,
assays during which the well contents require a fluid
media incubation phase or a bathing of the reactants
in fluid can be performed without errors or incon-
10 venience.

Another aspect of the present invention is the pendant
drop release fixture illustrated in Figs. 3 and 5.
This fixture is intended to be used with the multi-
15 chambered fluid collection means shown in Figs. 1 and,
4 which is designed to receive filtrate from the in-
terior of the well aligned directly above it via a
plurality of individual receiving chambers 50. In this
manner, the filtrate from each well will be retained
20 separately. This compartmentalization feature alone,
however, may not correct for the problem of comingling
of filtrates deriving from different wells as the
fluid is pulled through the hydrophobic fabric by an
applied differential pressure. Similary, in those
25 situations where the hydrophobic fabric is not present
or is not necessary for the purposes of the assay,
pendant drops will form and routinely collect on the
underside of each filtering area. In small volume
assays, the worker cannot afford to lose the 50 μ l
30 hanging as a drop from the membrane. Even in larger
volume assays, an accidental movement or subsequent
manipulations of the filter plate will dislodge the
pendant drop and cause it to fall into the wrong
receiving chamber causing cross-contamination of
35 filtrates and erroneous test results.

1 Both these kinds of problems are corrected by placement
of a pendant drop release fixture - in the form of a
guiding projection 60 - between the filtering area 28
5 and the fluid collection means 4 beneath the plate 20.
The preferred embodiment of this guiding projection 60
appears in Figs. 3 and 5 as a series of spikes 60
molded in a pattern corresponding to the individual
filtering areas 28 in the plate 20. Each spike 60 serves
10 a dual function: first, as a surface upon which the
small volumes of fluid which would otherwise be lost
as a pendant drop are collected and removed from the
filtering area 28; second, as a guide by which the fluids
forming a pendant drop are directed to the appropriate
15 chamber 50 in the fluid collection means 4. The pro-
jections 60 can be injection molded or a die cut
assembly. Any molding polymer material such as nylon,
polystyrene, polycarbonate and polyethylene may be used
for making the guiding projections; however, a hydrophilic
20 material is preferred because it promotes interception
and guidance of the pendant drop.

It is expected that the hydrophobic fabric and the
fluid guiding projection will be used in tandem in the
25 majority of assays. Nevertheless, where retention of
fluid within the well is not necessary, the pendant
drop release fixture may be used alone to advantage.

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1 **PATENTANWÄLTE**
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5 Millipore Corporation
 Bedford Massachusetts 01730
 U.S.A.

June 30, 1983

Eu 83 223 AS/K

10 Claims

1. A filtration apparatus comprising:

 a plate having at least one aperture open at each
 end;

15 filtration means disposed over one end of said
 aperture in said plate such that a well having a
 discrete filtering area is formed; and
 a hydrophobic fabric attached to said filtration
 means adjacent to said filtering area.

20

2. A filtration apparatus comprising:

 a plate having at least one aperture open at each
 end;

25 filtration means disposed over across one end of
 said aperture in said plate such that a well having
 a discrete filtering area is formed; and
 a projection aligned beneath said filtration means
 such that fluid passing through said filtering
 area is directed to a predetermined location.

30

3. A filtration apparatus comprising:

 a plate having at least one aperture open at each
 end;

35 filtration means disposed over one end of said
 aperture such that a well having a discrete filtering

- 1 area is formed;
a hydrophobic fabric attached to said filtration
means adjacent to said filtering area; and
a projection aligned beneath said filtration means
5 such that fluid passing through said filtering area
is directed to a predetermined location.
4. The filtration apparatus as recited in claim 1, 2
or 3 wherein said filtration means includes a
10 microporous membrane.
5. The filtration apparatus as recited in claim 4
wherein said filtration means includes a micro-
porous membrane having a porosity of at least 25
15 manometers.
6. The filter apparatus as recited in claim 1, 2 or 3
wherein said filtration means includes ultrafiltration
20 media.
7. The filtration apparatus as recited in claim 1 or 3
wherein said hydrophobic fabric is selected from the
group consisting of woven or nonwoven polymers.
- 25 8. The filter apparatus as recited in claim 1 or 3
wherein said hydrophobic fabric is selected from
the group consisting of polyesters, polyolefins and
polytetrafluoroethylene.
- 30 9. The filter apparatus as recited in claim 1 or 3
wherein said hydrophobic fabric is heat bondable.
10. The filter apparatus as recited in claim 1 or 3
35 wherein said hydrophobic fabric is attached with

1 adhesive.

11. The filtration apparatus as recited in claim 1, 2
5 or 3 further comprising fluid collections means
 disposed beneath said filtration means.

12. The filtration apparatus as recited in claim 11
 wherein said fluid collection means includes a
10 plurality of individual receiving chambers.

13. The filtration apparatus as recited in claim 2 or 3
 wherein said projection is disposed upon fluid
 collection means.

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Fig. 1

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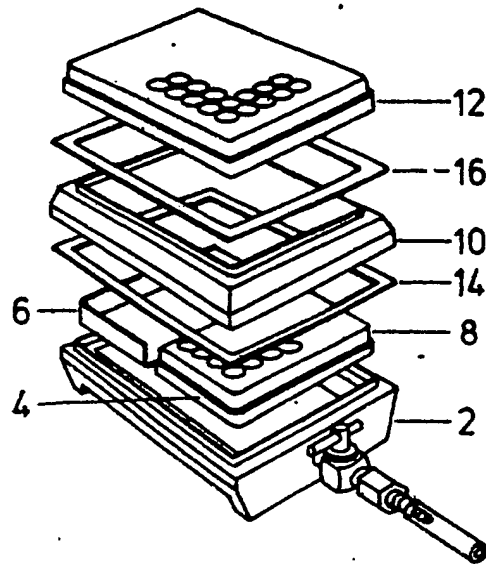


Fig. 2

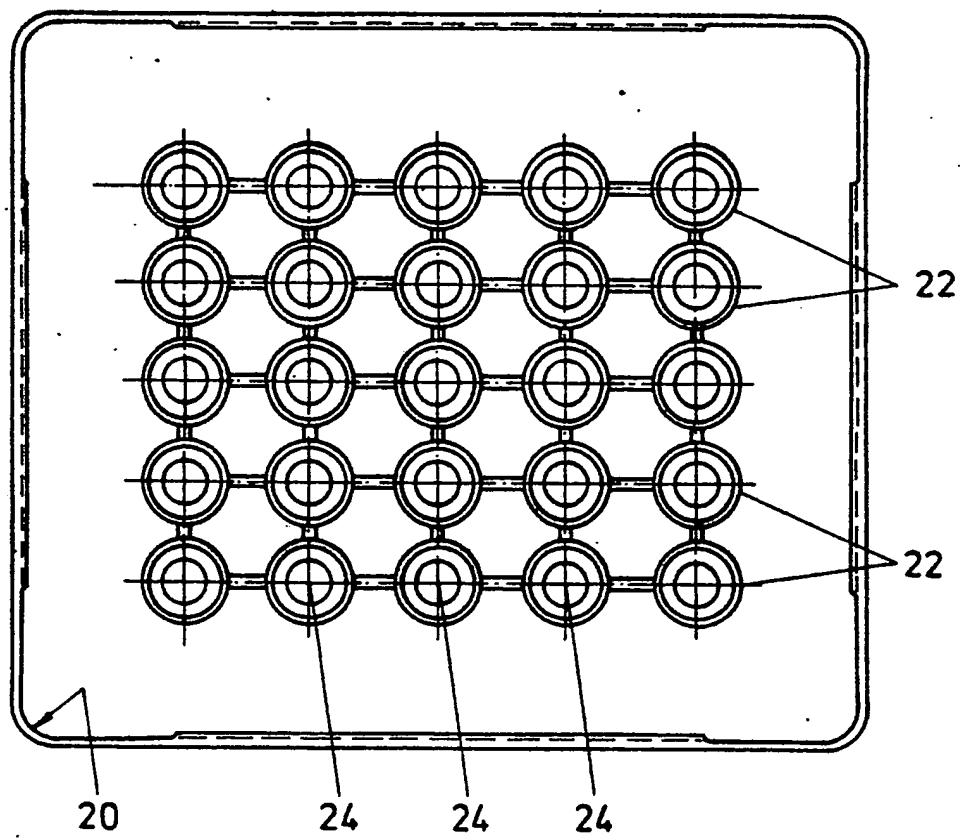


Fig. 3

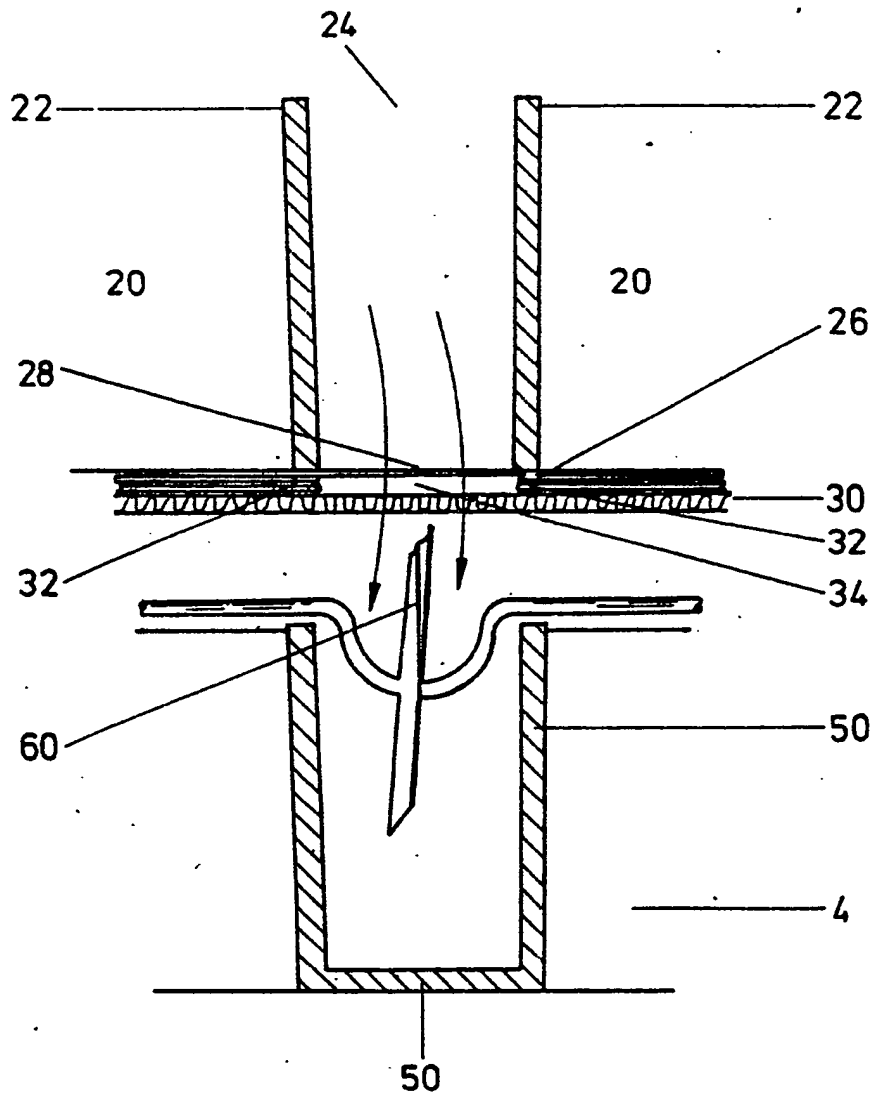


Fig. 4

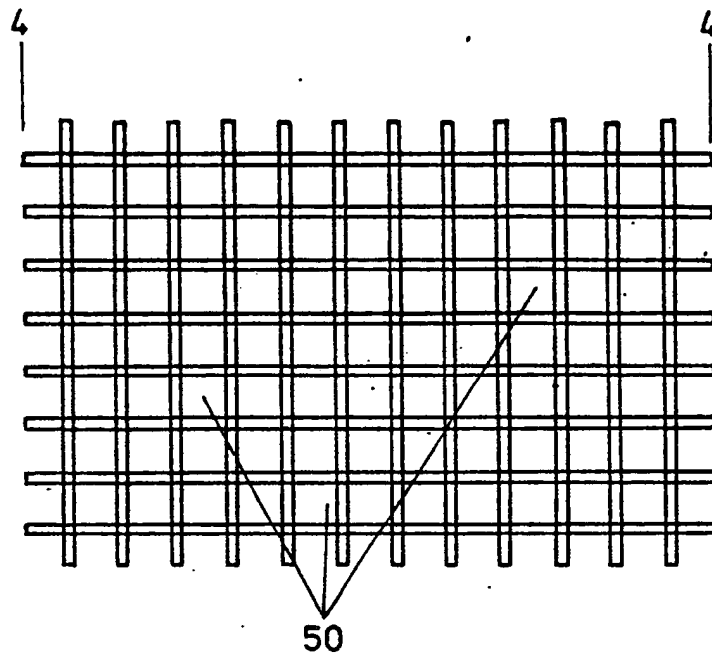
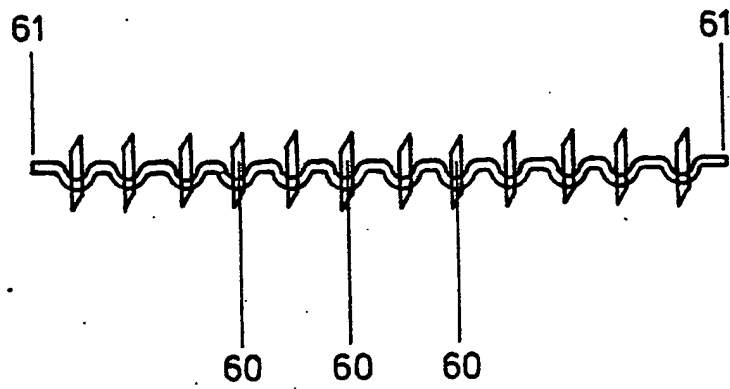


Fig. 5





European Patent
Office

EUROPEAN SEARCH REPORT

0098534

Application number

DOCUMENTS CONSIDERED TO BE RELEVANT			EP 83106390.4
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 3)
D,A	<u>US - A - 4 304 865</u> (O'BRIEN et al.) * Abstract * --	1	G 01 N 33/48 B 01 D 25/04
A	<u>US - A - 3 540 857</u> (D.N. MARTIN) * Claims 8,9 * --	1	
A	<u>US - A - 3 540 858</u> (J.E. ROCHTE et al.) * claims 1-4 * --	1	
A	<u>US - A - 3 540 856</u> (J.E. ROCHTE et al.) * Claims * --	1	
A	<u>US - A - 3 111 489</u> (A.R. GETZIN) * Claims * --	1	
A	<u>GB - A - 2 000 694</u> (TORAY INDUSTRIES INC.) * Abstract * ----	1,4-10	TECHNICAL FIELDS SEARCHED (Int. Cl. 3) B 01 D G 01 N 33/00 C 12 N
The present search report has been drawn up for all claims			
Place of search VIENNA		Date of completion of the search 27-09-1983	Examiner SCHNASS
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	